基于分子证据确认秦岭藤属与驼峰藤属(夹竹桃科)的系统 位置

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摘要: 秦岭藤属(Biondia Schltr.)是中国特有属,约 13 种,驼峰藤属(Merrillanthus Chun & Tsiang)是仅在我国与柬埔寨分布的单种属,目前两个属均已被归并到白前属(Vincetoxicum Wolf),但取样和系统发育分析尚存一些欠缺,其系统位置和归属尚需进一步确认。使用 2 个核糖体基因序列片段(ITS、ETS)数据、5 个叶绿体基因序列片段(psbA-trnH、trnG、trnL、trnL-F、trnT-L)数据,以及二者的合并数据,重建了娃儿藤亚族(Tylophorinae)的(包含属的模式秦岭藤[B. chinensis Schltr.=V. shaanxiense (Schltr.) Meve & Liede]与驼峰藤[M. hainanensis Chun & Tsiang=V. hainanense (Chun & Tsiang) Meve, H.H.Kong & Liede])系统发育树。结果表明:秦岭藤属与驼峰藤属均包含在白前属中,秦岭藤与青龙藤[B. henryi (Warb.) Tsiang & Li=V. henryi (Warb.) Meve & Liede]互为姐妹类群,并与 V. kawaroense Meve & Liede 聚为一支,而黑水藤[B. insignis Tsiang=V. insigne (Tsiang) Meve, H.H.Kong & Liede]在另一分支中(亚热带分支 Subtropical Clade);驼峰藤属与 V. cissoides (Blume) Kuntze 和 V. philippicum Meve, Omlor & Liede 聚为一支。核糖体和叶绿体基因数据均支持秦岭藤属与驼峰藤属归并入白前属,但秦岭藤属为多系,尚需收集更多种类和数据合并分析,深入探讨归并后的白前属的种间系统关系和位置。

关键词: 夹竹桃科,白前属,秦岭藤属,驼峰藤属,系统发育,中国中图分类号: Q949.7 **文献标识码**: A

Confirmation of the systematic position about *Biondia* and *Merrillanthus* (Apocynaceae) based on molecular evidence

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Abstract: Biondia Schltr., contains about 13 species, is endemic to China, and Merrillanthus

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Chun & Tsiang is a monotypic genus distributing only in China and Cambodia. These two genera have been included in *Vincetoxicum* Wolf., but their phylogenetic positions and taxonomic status need further study because of insufficient samples and phylogenetic analysis. We reconstructed the phylogenetic trees of Tylophorinae based on two nuclear ribosomal DNA data (ITS, ETS) and five plastid DNA data (*psbA-trnH*, *trnG*, *trnL*, *trnL-F*, *trnT-L*) individually and combined data, which contain two type species samples. The results showed that *Biondia* and *Merrillanthus* were nested inside the *Vincetoxicum*; *Biondia* was polyphyletic, and the type species *B. chinensis* Schltr.=*V. shaanxiense* (Schltr.) Meve & Liede was sister to *B. henryi* (Warb.) Tsiang & Li=*V. henryi* (Warb.) Meve & Liede, and formed a clade with *V. kawaroense* Meve & Liede, but *B. insignis* Tsiang=*V. insigne* (Tsiang) Meve, H.H.Kong & Liede belonged to the Subtropical Clade; *Merrillanthus* formed a clade with *V. cissoides* (Blume) Kuntze and *V. philippicum* Meve, Omlor & Liede. Our results support that *Biondia* and *Merrillanthus* should be included in *Vincetoxicum*, but *Biondia* is polyphyletic, more species samples and data need to be collected and analysed to confirm their systematic positions about *Vincetoxicum*.

Key words: Apocynaceae, Vincetoxicum, Biondia, Merrillanthus, phylogeny, China

夹竹桃科(Apocynaceae)在 Angiosperm Phylogeny Group IV 系统(APG IV)中与钩吻科(Gelsemiaceae)、龙胆科(Gentianaceae)、马钱科(Loganiaceae)和茜草科(Rubiaceae)共同属于龙胆目(Gentianales)(Chase et al., 2016),包含传统界定的狭义夹竹桃科(Apocynaceae s.s.)和萝藦科(Asclepiadaceae)两大类群,约 5350 种,主要分布在热带和亚热带地区(Endress et al., 2018)。夹竹桃科目前已建立了相对稳定的分子演化系统(Fishbein et al., 2018),一些修订的属也作了归并或位置调整,目前总属数由 422 个(Endress & Bruyns 2000)缩减到 378 个(Endress et al., 2018)。

白前属(Vincetoxicum Wolf)隶属于夹竹桃科马利筋族(Asclepiadeae)娃儿藤亚族(Tylophorinae),范围变化较大(Endress et al., 2018)。传统的白前属通常是茎直立,少数上部缠绕,须根簇生,花冠辐状,有 5 个肉质的副花冠裂片,有透明乳汁(邱声祥等,1989;Liede, 1996)。白前属与鹅绒藤属(Cynanchum L.)具有相似的副花冠、合蕊柱、花粉器等特征,部分学者认为白前属应该归并在鹅绒藤属中(蒋英和李秉滔,1977;Forster, 1991;Li et al., 1995),但也有学者认为白前属应该作为独立的属(Markgraf, 1972;Ali & Khatoon, 1982)。邱声祥等(1989)结合化学成分、形态、染色体数目和分布区等证据,提出白前属应该从鹅绒藤属中独立出来,Liede(1996)基于形态与化学证据也支持该观点,并认为其与娃儿藤属(Tylophora R. Br.)的关系最近。

然而,分子系统学研究显示白前属与鹅绒藤属亲缘关系较远(Liede, 2001; Rapini et al., 2007),支持白前属与娃儿藤属关系更近,但它们都是非单系的,而秦岭藤属(*Biondia* Schltr.)、*Blyttia* Arn.、*Diplostigma* K. Schum.、*Goydera* Liede、*Pleurostelma* Baill.和 *Rhyncharrhena* F. Muell.6 个属都嵌套在 *Vincetoxicum-Tylophora* 分支中(Liede-Schumann et al., 2012, 2016)。娃儿藤属和上述 6 个属,以及形态上相近的一些属均被归并在白前属中,增加了大量的新组合或新名称(Kidyoo & Kidyoo, 2018; Liede-Schumann & Meve, 2018; Hsu et al., 2021; Shah et al., 2021)。原主要分布在中国的 3 个属——驼峰藤属(*Merrillanthus* Chun & Tsiang)、白水藤属(*Pentastelma* Tsiang & Li)和箭药藤属(*Belostemma* Wall. ex Wight)也归并在白前属中。新定义的白前属有 150 种以上,通常有透明乳汁;花小,花冠辐状,少数钟状、坛状、长圆锥状;副花冠由肉质分离的雄蕊上的裂片组成,或由合生的雄蕊和片状的雄蕊间的部分组成环状;花粉块柄直立、水平或斜上,分布于热带、亚热带非洲、亚洲、欧亚大陆等区域(Endress et al., 2018)。

秦岭藤属约 13 种,是中国特有属,分布于我国东部和西南部;驼峰藤属仅有驼峰藤(Merrillanthus hainanensis Chun et Tsiang)1 种,分布于我国广东、海南与柬埔寨(蒋英和李秉滔,1977;Li et al., 1995)。在 Liede-Schumann 等(2012, 2016)的系统发育分析中,秦岭藤属仅包含 2 个种——黑水藤(Biondia insignis Tsiang)和青龙藤[Biondia henryi (Warb. ex Schltr. et Diels) Tsiang et P. T. Li]的样品,缺少属的模式秦岭藤(Biondia chinensis Schltr.)的样品,同时缺乏驼峰藤属的样品。部分学者报道了秦岭藤与驼峰藤的叶绿体基因组数据,并进行了简易的系统发育分析,其中秦岭藤显示与 V. rossicum (Kleopow) Barbar.是姐妹类群,但系统树中仅包含白前属的 1 个种的数据(Rao et al., 2018);驼峰藤则显示与黑水藤是姐妹类群,但系统发育树中的取样也太少(Xiong et al., 2019),这两个种是否属于白前属,或属于白前属哪个分支等问题依旧不清楚。

本研究收集了秦岭藤与驼峰藤的数据,其中新增驼峰藤的分子测序,秦岭藤的数据使用Rao等(2018)的测序结果,并结合白前属其他物种的分子数据(Liede-Schumann et al., 2016),开展了系统发育分析,拟进一步明确这2个属的系统位置和归属。

1 材料与方法

1.1 类群取样和分子序列数据来源

秦岭藤 (凭证标本: ZJB-2017-152-1,保存于陕西师范大学) 数据来源于 Rao 等 (2018) 中的浅层测序基因数据;对驼峰藤(凭证标本: LHB-AP17,保存于华南农业大学)进行叶 片取样, 经硅胶干燥后, 将样品放入干冰中冷藏, 快递至北京诺禾致源生物有限公司武汉分 公司进行基因组总 DNA 的提取、小片段文库的建库与测序。具体操作如下: DNA 经检测 合格后, 随机打断为 350 bp 左右的文库, 再进行 PE150 双端测序, 得到原始数据 (raw data), 经质控后得到最终的有效数据(clean data) 10Gb。将秦岭藤的已有浅层测序数据与本次测 序的驼峰藤的数据用 GetOrganelle1.7 (Jin et al., 2020) 进行组装,设置参数为默认:使用 Bandage 0.8. (Wick et al., 2015) 和 Geneious Prime 2019 (https://www.geneious.com/) 对组装 得到的 fastg 文件进行可视化和序列提取,得到最终的秦岭藤的核糖体基因组序列,以及驼 峰藤的叶绿体基因组与核糖体基因组序列。分别以无油樟[Amborella trichopoda Baill. (AJ506156)]和罗布麻[Apocynum venetum L. (MT313688)]的叶绿体基因组作参考,利用 Plastid Genome Annotator (PGA) (Qu et al., 2019) 对驼峰藤的叶绿体基因组进行注释,再 根据 log 文档在 Geneious Prime 2019 中进行进一步的手动校正,得到叶绿体基因组的注释信 息; 以 Asclepias coulteri A.Gray (JN665084) 的核糖体基因组 nrDNA 连续片段 (18S+ITS1+5.8S+ITS2+26S) 和 V. biglandulosum (Endl.) Kuntze (LN880610) 的核糖体基因 序列片段(ETS)为参考,在 Geneious Prime 2019 中对秦岭藤与驼峰藤的核糖体基因组进行 注释,并利用 Excract 选项提取出所需的核糖体基因序列片段与叶绿体基因序列片段,并将 数据上传至 GenBank(https://www.ncbi.nlm.nih.gov/)。

基于已发表的娃儿藤亚族分子系统学研究数据(Liede-Schumann et al., 2016),补充驼峰藤和秦岭藤的 DNA 数据,选取鹅绒藤亚族(Cynanchinae)中的 3 个种为外类群,分别利用 5 个叶绿体基因序列片段(psbA-trnH、trnG、trnL、trnL-F、trnT-L)(137 个种)、2 个核糖体基因序列片段(ETS、ITS)(136 个种),以及二者的合并数据构建系统发育树(139个种)。序列详细信息见表 1,样品凭证标本详细信息见(Liede-Schumann et al., 2016)。部分样品序列数据不全,相关序列矩阵中以缺失数据形式补齐。

1.2 序列比对和拼接

采用 MAFFT 软件(Katoh & Standley, 2013)单独对每个片段进行序列比对,多片段数据在 Geneious Prime 2019 中进行序列拼接,得到联合矩阵数据。

1.3 系统发育分析

利用 IQ-TREE (Nguyen et al., 2015) 基于最大似然法(maximum likelihood method, ML)对序列进行系统发育分析,IQ-TREE 中 ModelFinder 按照 BIC 准则(Bayesian Information Criterion, BIC)自动测试,并选择出最佳替代模型,其中核糖体基因序列片段合并数据使用模型为 TVM+F+R3、叶绿体基因序列片段合并数据使用模型为 K3Pu+F+R3、核糖体与叶绿体基因序列片段合并数据使用模型为 TVM+F+R3,再进行 1 000 次的 SH-aLRT 检验(Guindon et al., 2010)和超快自展值检验(ultrafast bootstrap approach, UFBoot)(Minh et al., 2013),每个分支含有 SH-aLRT 和 UFBoot 2 个支持率,如果 SH-aLRT >= 80%且 UFboot >= 95%,则视为得到较好支持,其分支结果可信,最后将所得系统树在 Figtree 1.4.2(Rambaut, 2012)中查看。

表 1 取样物种和序列 GenBank 编号 Table 1 List of taxa sampled and GenBank accession numbers of sequences

类群	类群原先使用名称		tC	4 I	4I. E	trnT–L	ETC	ITC
Taxon	Taxon name originally used	psbA–trnH	trnG	trnL	trnL–F	trn1–L	ETS	ITS
外类群 Outgroups								
鹅绒藤亚族 Cynanchinae								
Cynanchum ellipticum (Harv.)	C. ellipticum (Harv.) R.A.Dyer	HE802679	HE793818	AJ290846	AJ290845	AJ290847	_	AJ320444
R.A.Dyer	C. empucum (Harv.) K.A.Dyer	HE8020/9	HE/93818	AJ290840	AJ290843	AJ29084 /	_	AJ320444
台湾牛皮消 C. ovalifolium Wight	台湾牛皮消 C. ovalifolium Wight	HE802680	HE793819	AJ428697	AJ428698	AJ428696	_	AJ492780
Schizostephanus alatus Hochst. ex	С. 1. И. 1. И.С.1	HE902/79	HE793827	AJ410248	AJ410249	AJ410247		A 1220451
K.Schum.	S. alatus Hochst. ex K.Schum.	HE802678	ПЕ/9362/	AJ410246	AJ410249	AJ41024/	_	AJ320451
内类群 Ingroups								
娃儿藤亚族 Tylophorinae								
Pentatropis bentii (N.E. Br.) Liede a	P. bentii (N.E. Br.) Liede a	LN880644	LN880691	_	LN880737	LN880780	LN880601	LN880556
P. bentii (N.E. Br.) Liede b	P. bentii (N.E. Br.) Liede b	LN880645	LN880692	_	LN880738	LN880781	LN880602	LN880557
P. capensis (L.f.) Bullock	P. capensis (L.f.) Bullock	LN880646	LN880693	_	LN880739	LN880782	LN880603	LN880558
P. madagascariensis Decne.	P. madagascariensis Decne.	HE793884	HE793823	AJ410236	AJ410237	AJ410235	HE793968	AJ320448
P. nivalis (J.F. Gmel.) D.V. Field &	P. nivalis (J.F. Gmel.) D.V. Field &	HE793885	HE793824	AJ410239	AJ410240	AJ410238	HE793969	AJ320449
J.R.I. Wood a	J.R.I. Wood a	HE/93003	11E/93024	AJ410239	AJ410240	AJ410238	11L/93909	AJ340 44 9
P. nivalis (J.F. Gmel.) D.V. Field &	P. nivalis (J.F. Gmel.) D.V. Field &	HE793852	HE793784	HE793746	HE793755	HE793717	HE793939	HE793910
J.R.I. Wood b	J.R.I. Wood b	HE/93032	11E/93/04	11E/35/40	1111/93/33	11E/93/1/	11E/23239	11E/9391U

Pentatropis sp.	Pentatropis sp.	LN880648	LN880695	_	LN880741	LN880784	LN880605	LN880560
P. spiralis Decne.	P. spiralis Decne.	LN880647	LN880694	_	LN880740	LN880783	LN880604	LN880559
Vincetoxicum aff. rotundifolium (BuchHam. ex Wight) Kuntze	Tylophora aff. rotundifolia BuchHam. ex Wight	HE793866	HE793798	_	HE793769	HE793731	HE793950	НЕ793924
V. ambiguum Maxim.	V. ambiguum Maxim.	AB109146	AB109087	AB109928	AB109956	_	AB110047	AB109984
V. anomalum (N.E.Br.) Meve & Liede	T. anomala N.E. Br. a	HE793887	HE793829	AJ410251	AJ410252	AJ410250	HE793971	AJ320452
V. anomalum (N.E.Br.) Meve & Liede b	T. anomala N.E. Br. b	HE793855	HE793787	_	HE793758	HE793720	HE793942	HE793913
V. anomalum (N.E.Br.) Meve & Liede c	T. anomala N.E. Br. c	LN880649	LN880696	LN880742	_	LN880785	LN880606	LN880561
V. anomalum (N.E.Br.) Meve & Liede d	T. anomala N.E. Br. d	LN880650	LN880697	_	LN880743	LN880786	LN880607	LN880562
V. anomalum (N.E. Br.) Meve & Liede	T. urceolata Meve	HE793867	HE793799	_	HE793770	HE793732	HE793951	HE793925
V. apiculatum (K.Schum.) Meve & Liede a	T. apiculata K.Schum a	LN880651	LN880698	_	LN880744	LN880787	LN880608	LN880563
V. apiculatum (K.Schum.) Meve & Liede b	T. apiculata K.Schum b	HE793888	HE793830	AJ410254	AJ410255	AJ410253	_	AJ320453
V. apiculatum (K.Schum.) Meve & Liede c	T. apiculata K.Schum c	LN880652	LN880699	_	LN880745	LN880788	LN880609	LN880564
V. arachnoideum (Goyder) Meve & Liede	T. arachnoidea Goyder	HE793856	HE793788	_	HE793759	HE793721	HE793943	HE793914

V. aristolochioides (Miq.) Franch. & Sav.	T. aristolochioides Miq.	AB109137	AB109078	AB109912	AB109944	_	AB110038	AB109975
V. arnottianum Wight	V. arnottianum Wight	LN880662	LN880709	_	LN880752	LN880797	LN880616	LN880574
V. assadii M. Zaeifi	V. assadii M. Zaeifi	LN880663	LN880710	_	LN880753	LN880798	LN880617	LN880575
V. austrokiusianum (Koidz.) Kitag.	V. austrokiusianum (Koidz.) Kitag.	AB109149	AB109117	AB109927	AB109959	_	AB110050	AB109987
V. barbatum (R. Br.) Kuntze	T. barbata R. Br.	HE793857	HE793789	_	HE793760	HE793722	_	HE793915
V. biglandulosum (Endl.) Kuntze a	T. biglandulosa F. Muell. a	LN880653	LN880700	_	LN880746	LN880789	LN880610	LN880565
V. biglandulosum (Endl.) Kuntze b	T. biglandulosa F. Muell. b	HE793889	HE793831	AJ320402	AJ320403	AJ320401	_	AJ320454
V. brachystelmoides (P.I. Forst.) Liede	V. brachystelmoides (P.I. Forst.) Liede	LN880664	LN880711	_	LN880754	LN880799	LN880618	LN880576
V. caffrum (Meisn.) Kuntze	T. caffra Meisn.	HE793858	HE793790	HE793749	HE793761	HE793723	HE793944	HE793916
V. calcareum (H. Ohashi) Akasawa	V. calcareum (H. Ohashi) Akasawa	AB109150	AB109118	AB109929	AB109960	_	AB110051	AB109988
V. cameroonicum (N.E. Br.) Meve & Liede	T. cameroonica N.E. Br.	HE793859	HE793791	_	HE793762	HE793724	HE793945	HE793917
V. carnosum Benth. a	V. carnosum Benth. a	LN880666	LN880713	_	LN880756	LN880801	LN880620	LN880578
V. carnosum Benth. b	V. carnosum Benth. b	HE793905	HE793847	AJ410272	AJ410273	AJ410271	HE793986	AJ320473
V. cernuum (Decne.) Meve & Liede	Pleurostelma cernuum (Decne.) Bullock	HE793886	HE793826	AJ410242	AJ410243	AJ410241	HE793970	AJ320450
V. cissoides (Blume) Kuntze	T. cissoides Blume	HE793860	HE793792	_	HE793763	HE793725	_	HE793918
V. confusum Meve & Liede	T. coriacea (Decne.) Marais	HE793891	HE793833	AJ320408	AJ320409	AJ320407	HE793972	AJ320456
V. conspicuum (N.E. Br.) Meve & Liede	T. conspicua N.E. Br.	HE793890	HE793832	AJ320405	AJ320406	AJ320404	_	AJ320455

V. creticum Browicz	V. creticum Browicz	HE793871	HE793803	_	HE793774	HE793736	HE793955	HE793928
V. dalzellii (Hook.f.) Kuntze	T. dalzellii Hook.f.	LN880654	LN880701	_	HG530585	LN880790	_	LN880566
V. diplostigma Meve & Liede	Diplostigma canescens K. Schum.	HE793882	HE793820	AJ410200	AJ410201	AJ410199	HE793966	AJ320445
V. flanaganii (Schltr.) Meve & Liede	T. flanaganii Schltr.	HE793892	HE793834	AJ410257	AJ410258	AJ410256	HE793973	AJ320457
V. fleckii (Schltr.) Meve & Liede	T. fleckii N.E. Br.	LN880655	LN880702	_	LN880747	LN880791	LN880611	LN880567
V. flexuosum (R.Br.) Kuntze var.								
perrottetianum (Decne.) Schneidt,	T. perrottetiana Decne.	HE793898	HE793840	AJ290916	AJ290917	AJ290915	HE793979	AJ320460
Meve & Liede								
V. flexuosum (R. Br.) Kuntze var.								
tenuis (Blume) Schneidt, Meve &	T. tenuis Blume	HE793901	HE793843	AJ320432	AJ320433	AJ320431	HE793982	AJ320468
Liede								
V. fruticulosum (Decne.) Decaisne	Blyttia fruticulosa (Decne.) Field	HE793881	HE793815	AJ410194	AJ410195	AJ410193	HE793965	AJ320443
V. funebre (Boiss. & Kotschy) Pobed.	V. funebre (Boiss. & Kotschy) Pobed. a	LN880667	LN880714	_	LN880757	LN880802	LN880621	LN880579
V. funebre (Boiss. & Kotschy) Pobed.	V. funebre (Boiss. & Kotschy) Pobed. b	LN880668	LN880715	_	LN880758	LN880803	LN880622	LN880580
V. fuscatum (Hornem.) Endl.	V. fuscatum (Hornem.) Endl.	LN880669	LN880716	_	LN880759	LN880804	LN880623	_
V. glaucum (Wall. ex Wight) Rech.f.	V. glaucum (Wall. ex Wight) Rech.f.	LN880670	LN880717	_	LN880760	LN880805	LN880624	LN880581
V. gracillimum (Markgr.) Meve & Liede	T. gracillima Markgr.	HE793861	HE793793	HE793750	HE793764	HE793726	HE793946	HE793919
V. grandiflorum (R. Br.) Kuntze a	T. grandiflora R. Br. a	HE793862	HE793794	HE793765	_	HE793727	HE793947	HE793920
V. grandiflorum (R. Br.) Kuntze b	T. grandiflora R. Br. b	LN880657	LN880704	LN880748	_	LN880792	_	LN880569
V. heterophyllum (A. Rich.) Vatke	T. heterophylla A. Rich.	HE793894	HE793836	AJ410260	AJ410261	AJ410259	HE793975	AJ320461
V. hoyoense T. Yamash.	V. hoyoense T. Yamash.	AB109164	AB109132	AB109940	AB109971	_	AB110065	AB110003

V. indicum (Burm.f.) Mabb.	T. indica (Burm.f.) Merrill	HE793895	HE793837	AJ410263	AJ410264	AJ410262	HE793976	AJ320463
V. intermedium Taliev	V. intermedium Taliev	LN880672	LN880719	_	LN880762	LN880807	LN880626	LN880583
V. izuense T. Yamash.	V. izuense T. Yamash.	AB109163	AB109131	AB109939	AB109970	_	AB110064	AB110002
V. jailicola Juz.	V. jailicola Juz.	LN880673	LN880720	_	LN880763	LN880808	LN880627	LN880584
V. japonicum Morr. et Decne.	V. japonicum Morr. et Decne.	AB109151	AB109119	AB109930	AB109961	_	AB110052	AB109990
V. katoi (Ohwi) Kitag.	V. katoi (Ohwi) Kitag.	AB109152	AB109120	AB109931	AB109962	_	AB110053	AB109991
V. kawaroense Meve & Liede	T. japonica Miq.	AB109140	AB109081	AB109915	AB109947	_	AB110041	AB109978
V. lineare (Decne.) Meve & Liede a	Rhyncharrhena linearis (Decne.) K. L. Wilson a	HE793853	HE793785	HE793747	HE793756	HE793718	HE793940	HE793911
V. lineare (Decne.) Meve & Liede b	R. linearis (Decne.) K. L. Wilson b	HE793854	HE793786	HE793748	HE793757	HE793719	HE793941	HE793912
V. linifolium Balfour f.	V. linifolium Balfour f.	LN880674	LN880721	_	LN880764	LN880809	LN880628	LN880585
V. lugardiae (Bullock) Meve & Liede,	T. lugardiae Bullock	HE793864	HE793796	HE793751	HE793767	HE793729	HE793949	HE793922
V. lycioides (E. Mey.) Kuntze	T. lycioides Decne.	LN880658	LN880705	LN880749	_	LN880793	LN880613	LN880570
V. macrophyllum Sieb. et Zucc. a	V. macrophyllum Sieb. et Zucc. a	AB109155	AB109122	AB109922	AB109954	_	AB110054	AB109993
V. macrophyllum Sieb. et Zucc. b	V. macrophyllum Sieb. et Zucc. b	AB109154	AB109121	AB109921	AB109953	_	AB110055	AB109992
V. maeoticum (Kleopow) Barbar.	V. maeoticum (Kleopow) Barbar.	LN880675	LN880722	_	LN880765	LN880810	LN880629	LN880586
V. magnificum (Nakai) Kitag.	V. magnificum (Nakai) Kitag.	AB109156	AB109123	AB109923	AB109955	_	AB110056	AB109994
V. matsumurae (T. Yamaz.) H.Ohashi	T. matsumurae (T. Yamaz.)T. Yamash. & Y. Tateishi	AB109143	AB109082	AB109916	AB109948	_	AB110042	AB109979

V. mozaffarianii M. Zaeifi	V. mozaffarianii M.Zaeifi	LN880676	LN880723	_	LN880766	LN880811	LN880630	LN880587
V. nigrum (L.) Moench a	V. nigrum (L.) Moench a	HE793907	HE793849	_	_	_	HE793988	_
V. nigrum (L.) Moench b	V. nigrum (L.) Moench b	_	_	_	_	AY899964	_	_
V. nigrum (L.) Moench c	V. nigrum (L.) Moench c	_	_	AF214451	AF214297	_	_	_
V. nigrum (L.) Moench d	V. nigrum (L.) Moench d	_	_	_	_	_	_	FJ362532
V. nipponicum (Matsum.) Kitag. a	V. nipponicum (Matsum.) Kitag. a	AB109158	AB109125	AB109933	AB109964	_	AB110058	AB109996
V. nipponicum (Matsum.) Kitag. b	V. nipponicum (Matsum.) Kitag. b	AB109157	AB109124	AB109932	AB109963	_	AB110057	AB109995
V. oblongum (N.E. Br.) Meve & Liede	T. oblonga N.E. Br.	HE793896	HE793838	AJ320423	AJ320424	AJ320422	HE793977	AJ320464
V. ovatum Benth.	V. ovatum Benth.	HE793875	HE793807	_	HE793778	HE793740	HE793959	HE793932
V. paniculatum (R. Br.) Kuntze	T. paniculata R. Br.	HE793865	HE793797	HE793768	_	HE793730	_	HE793923
V. philippicum Meve, Omlor & Liede	T. parviflora (Merr.) Meve, Omlor & Liede	HE793897	HE793839	AJ320426	AJ320427	AJ320425	HE793978	AJ320465
V. pumilum Decne. a	V. pumilum Decne. a	LN880677	LN880724	_	LN880767	LN880812	LN880631	LN880588
V. pumilum Decne. b	V. pumilum Decne. b	LN880678	LN880725	_	LN880768	LN880813	LN880632	LN880589
V. rehmannii Boiss. a	V. rehmannii Boiss. a	LN880679	LN880726	_	LN880769	LN880814	LN880633	LN880590
V. rehmannii Boiss. b	V. rehmannii Boiss. b	LN880680	LN880727	_	LN880770	LN880815	LN880634	LN880591
V. rossicum (Kleopow) Barbar. a	V. rossicum (Kleopow) Barbar. a	_	HE793809	_	_	HE793742	HE793990	_
V. rossicum (Kleopow) Barbar. b	V. rossicum (Kleopow) Barbar. b	_		_	EF456113	_	_	_
V. rossicum (Kleopow) Barbar. c	V. rossicum (Kleopow) Barbar. c	_	_	_	_	_	_	FJ517165

V. sakesarense Ali & Khatoon	V. sakesarense Ali & Khatoon	LN880682	_	_	_	LN880816	LN880635	LN880592
V. scandens Sommier & Levier a	V. scandens Sommier & Levier a	LN880683	LN880729	_	LN880772	LN880817	LN880636	LN880593
V. scandens Sommier & Levier b	V. scandens Sommier & Levier b	LN880684	LN880730	_	LN880773	LN880818	LN880637	LN880594
V. schmalhausenii (Kusn.) Litv.	V. schmalhausenii (Kusn.) Litv.	LN880685	LN880731	_	LN880774	LN880819	LN880638	LN880595
V. somaliense (Liede) Meve & Liede	Goydera somaliense Liede	HE793883	HE793821	AJ410209	AJ410210	AJ410208	НЕ793967	AJ320447
Vincetoxicum sp. a	Vincetoxicum sp. a	LN880689	LN880735	_	LN880778	LN880823	LN880642	LN880599
Vincetoxicum sp. b	Vincetoxicum sp. b	LN880690	LN880736	_	LN880779	LN880824	LN880643	LN880600
V. stocksii Ali & Khatoon	V. stocksii Ali & Khatoon	HE793908	HE793850	AJ410278	AJ410279	AJ410277	HE793989	AJ320475
V. sublanceolatum (Miq.) Maxim. var. sublanceolatum	V. sublanceolatum (Miq.) Maxim. var. sublanceolatum	AB109159	AB109127	AB109935	AB109966	_	AB110060	AB109998
V. sylvaticum (Decne.) Kuntze	T. sylvatica Decne.	HE793899	HE793841	AJ410266	AJ410267	AJ410265	HE793980	AJ320466
V. tanakae (Maxim.) Franch. & Sav.	T. tanakae Maxim.	AB109144	AB109085	AB109919	AB109951	_	AB110045	AB109982
V. tauricum Pobed.	V. tauricum Pobed.	LN880686	LN880732	_	LN880775	LN880820	LN880639	LN880596
V. tenuipedunculatum (K.Schum.) Meve & Liede	T. tenuipedunculata K. Schum.	HE793900	HE793842	AJ320429	AJ320430	AJ320428	HE793981	AJ320467
V. tmoleum Boiss.	V. tmoleum Boiss.	LN880687	LN880733	_	LN880776	LN880821	LN880640	LN880597
V. villosum (Blume) Kuntze a	T. villosa Blume a	HE793902	HE793844	AJ320435	AJ320436	AJ320434	HE793983	AJ320469
V. villosum (Blume) Kuntze b	T. villosa Blume b	HE793868	HE793800	_	HE793771	HE793733	HE793952	HE793926
V. yamanakae (Ohwi & H. Ohashi) H. Ohashi	V. yamanakae (Ohwi & H. Ohashi) H. Ohashi	AB109153	AB109129	AB109937	AB109968	_	AB110062	AB110000

V. yonakuniense (Hats.) T. Yamash. & Y. Tateishi	V. yonakuniense (Hats.)T. Yamash. & Y. Tateishi	AB109162	AB109130	AB109938	AB109969	_	AB110063	AB110001
白前 V. glaucescens (Decne.) C.Y. Wu & D.Z. Li	白前 V. glaucescens (Decne.) C.Y. Wu & D.Z. Li	HE793873	HE793805	HE793753	НЕ793776	HE793738	HE793957	HE793930
白薇 V. atratum C. Morren & Decne. a	白薇 V. atratum C. Morren & Decne. a	HE793904	HE793846	AJ410269	AJ410270	AJ410268	HE793985	AJ320472
白薇 V. atratum C. Morren & Decne. b	白薇 V. atratum C. Morren & Decne. b	AB109148	AB109089	AB109926	AB109958	_	AB110049	AB109986
变色白前 V. versicolor (Bunge) Decne.	变色白前 V. versicolor (Bunge) Decne.	HE793879	HE793812	_	HE793782	HE793745	HE793963	НЕ793936
潮风草 V. acuminatum Decne. a	潮风草 V. acuminatum Decne. a	AB109145	AB109086	AB109924	AB110215	_	AB110046	AB109983
潮风草 V. acuminatum Decne. b	潮风草 V. acuminatum Decne. b	LN880661	LN880708	_	LN880751	LN880796	LN880615	LN880573
催吐白前 V. hirundinaria Medic.	催吐白前 V. hirundinaria Medic.	HE793906	HE793848	AJ410275	AJ410276	AJ410274	HE793987	AJ320474
大理白前 <i>V. forrestii</i> (Schltr.) C.Y. Wu & D.Z. Li	大理白前 <i>V. forrestii</i> (Schltr.) C.Y. Wu & D.Z. Li	HE793872	HE793804	HE793752	HE793775	HE793737	HE793956	HE793929
粉绿白前 V. canescens (Willd.) Decne.	粉绿白前 V. canescens (Willd.) Decne.	LN880665	LN880712	_	LN880755	LN880800	LN880619	LN880577
光叶娃儿藤 <i>V. brownii</i> (Hayata) Meve & Liede	光叶娃儿藤 T. brownii Hayata	AB109138	AB109079	AB109913	AB109945	_	AB110039	AB109976
合掌消 V. amplexicaule Siebold et Zucc.	合掌消 V. amplexicaule Siebold et Zucc.	AB109147	AB109088	AB109925	AB109957	_	AB110048	AB109985
黑水藤 V. insigne (Tsiang) Meve, H.H.Kong & Liede	黑水藤 Biondia insignis Tsiang	HE793851	HE793783	_	HE793754	НЕ793716	HE793938	HE793909

华北白前 V. mongolicum Maxim.	华北白前 V. mongolicum Maxim.	HE793874	HE793806	_	HE793777	HE793739	HE793958	HE793931
柳叶白前 <i>V. stauntonii</i> (Decne.) C.Y. Wu & D.Z. Li	柳叶白前 <i>V. stauntonii</i> (Decne.) C.Y. Wu & D.Z. Li	HE793877	HE793810	_	HE793780	HE793743	HE793961	HE793934
蔓白前 V. volubile (Maxim.) Hemsl.	蔓白前 V. volubile (Maxim.) Hemsl.	LN880688	LN880734	_	LN880777	LN880822	LN880641	LN880598
蔓剪草 V. chekiangense (M. Cheng) C.Y. Wu & D.Z. Li	蔓剪草 <i>V. chekiangense</i> (M. Cheng) C.Y. Wu & D.Z. Li	HE793870	HE793802	_	НЕ793773	HE793735	HE793954	_
七层楼 V. floribundum (Miq.) Franch. & Sav.	七层楼 T. floribunda Miq.	AB109139	AB109080	AB109914	AB109946	_	AB110040	AB109977
秦岭藤 V. shaanxiense Meve & Liede	秦岭藤 Biondia chinensis Schltr.	MH210646	MH210646	MH210646	MH210646	MH210646	OL597536	OL442775
青龙藤 <i>V. henryi</i> (Warb. ex Schltr. & Diels) Meve & Liede	青龙藤 <i>Biondia henryi</i> (Warb. ex Schltr. & Diels) Tsiang & P.T. Li	HE793880	HE793814	AJ410191	AJ410192	AJ410190	HE793964	НЕ793937
驼峰藤 V. hainanense (Chun & Tsiang) Meve, H. H. Kong & Liede	驼峰藤 Merrillanthus hainanensis Chun et Tsiang	OL442775	OL442775	OL442775	OL442775	OL442775	OL597537	OL442774
娃儿藤 V. hirsutum (Wall.) Kuntze	娃儿藤 T. hirsuta (Wall.) Wight	HE793863	HE793795	_	HE793766	HE793728	HE793948	HE793921
娃儿藤 V. hirsutum (Wall.) Kuntze	娃儿藤 T. mollissima Wall. ex Wight	LN880659	LN880706	HG530586	_	LN880794	_	LN880571
娃儿藤 V. hirsutum (Wall.) Kuntze	娃儿藤 <i>T. ovata</i> (Lindl.) Hook. ex Steud.	AB109142	AB109084	AB109918	AB109950	_	AB110043	AB109981
小叶娃儿藤 <i>V. flexuosum</i> (R. Br.) Kuntze a	小叶娃儿藤 T. flexuosa R. Br. a	LN880656	LN880703	AJ320411	AJ320412	AJ320410	LN880612	LN880568

小叶娃儿藤 <i>V. flexuosum</i> (R. Br.) Kuntze b	小叶娃儿藤 T. flexuosa R. Br. b	HE793893	HE793835	AJ320414	AJ320415	AJ320413	HE793974	AJ320458
徐长卿 V. pycnostelma Kitag. a	徐长卿 V. pycnostelma Kitag. a	AB109161	AB109126	AB109934	AB109965	_	AB110059	AB109997
徐长卿 V. pycnostelma Kitag. b	徐长卿 V. pycnostelma Kitag. b	HE793876	HE793808	_	HE793779	HE793741	HE793960	HE793933
云南娃儿藤 V. yunnanense (Schltr.) Meve & Liede	云南娃儿藤 T. yunnanensis Schltr.	HE793903	HE793845	AJ320441	AJ320442	AJ320440	HE793984	AJ320471
竹灵消 V. inamoenum Maxim. a	竹灵消 V. inamoenum Maxim. a	AB109165	AB109133	AB109920	AB109952	_	AB110066	AB109989
竹灵消 V. inamoenum Maxim. b	竹灵消 V. inamoenum Maxim. b	LN880671	LN880718	_	LN880761	LN880806	LN880625	LN880582

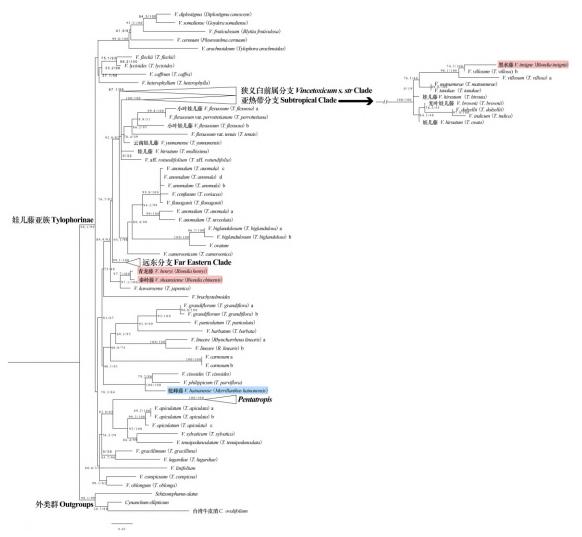
备注:一表示信息的缺失;加粗字体表示为新测物种,其中叶绿体基因组序列片段对应的序列号提供的是完整叶绿体基因组序列号,所用片段从中进行了截取。

Note: — indicates the absence of information; Taxa in bold are newly sequenced for the present study, the GenBank accession numbers corresponding to the plastid DNA data are provided for the complete chloroplast genome, and the plastid DNA data used were extracted from the complete chloroplast genome.

2 结果与分析

基于核糖体基因序列片段(ETS、ITS)构建的系统发育树显示娃儿藤亚族中白前属是非单系的,*Pentatropis* R. Br. ex Wight & Arn. 嵌套在白前属中,并与 *V. apiculatum* + *V. sylvaticum* + *V. tenuipedunculatum* 成姐妹分支,支持率分别为 SH-aLR = 82.8%、UFboot = 82% (82.8/82)(图 1); 基于叶绿体基因序列片段和合并数据构建的系统树都显示娃儿藤亚族分为 2 个大分支,*Pentatropis* R. Br. ex Wight & Arn. 与白前属呈姐妹关系,支持率分别为 99.8/100、99.9/100。

3 组分子矩阵数据所建系统树都高度支持驼峰藤属与 V. cissoides (T. cissoides) 和 V. Philippicum (T. parviflora) 聚为一支,图 1、图 2、图 3 中支持率分别为 100/100、97.9/100、100/100;秦岭藤属中的 3 个种均嵌套在白前属分支中,但在系统树中非单系,其中在基于核糖体基因序列片段(ETS、ITS)所建的系统树与合并数据所建的系统树中,秦岭藤与青龙藤 V. henryi(Biondia henryi)互为姐妹类群,支持率分别为 97.7/100、97.8/100(图 1、图 3),并与 V. kawaroense (T. japonica) 聚为一支,支持率分别为 97.1/100、97.8/100,在基于叶绿体基因序列片段构建的系统树中,秦岭藤与青龙藤聚为一支,但支持率较低(31.3/67)(图 2),而黑水藤在 3 组系统树中均显示在亚热带分支(Subtropical Clade)中(图 1、图 2、图 3 中支持率分别为 100/100、93.8/100、100/100),并与 V. villosum(T. villosa)聚为一支(图 1、图 2、图 3 中支持率分别为 74.3/100、0/96、72.6/100)。



分支节点数据代表 SH-aLRT 和 UFBoot 2 个支持率, SH-aLRT >= 80 且 UFboot >= 95, 视为结果可信, 分支的划分参照 Liede-Schumann 等(2016)。下同。

Each branch assigned with SH-aLRT and UFBoot supports, if SH-aLRT >= 80 and UFboot >= 95, the result is considered to be credible, clade designations follow those of Liede-Schumann et al. (2016). The same below.

图 1 基于 2 个核糖体基因序列片段(ETS、ITS)构建的娃儿藤亚族的系统发育树 Fig.1 Phylogeny tree of Tylophorinae constructed by two ribosomal gene sequences (ETS, ITS)

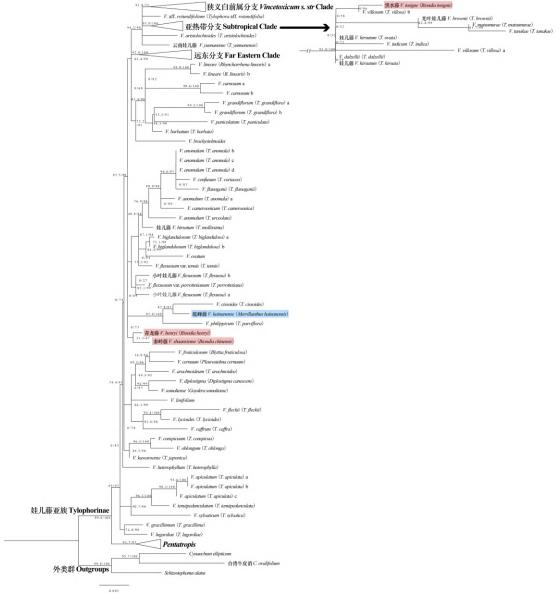


图 2 基于 5 个叶绿体基因序列片段(*psbA-trnH、trnG、trnL、trnL-F、trnT-L*)构建的娃儿藤亚族的系统发育树

Fig.2 Phylogeny tree of Tylophorinae constructed by five plastid markers (*psbA–trnH*, *trnG*, *trnL*, *trnL–F*, *trnT–L*)

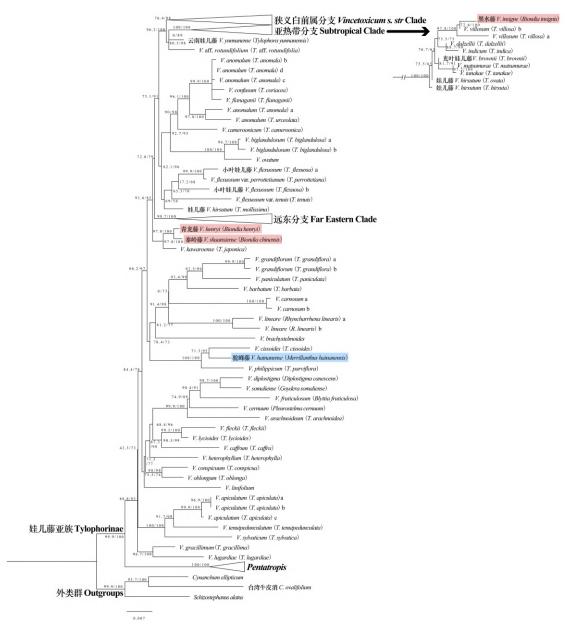


图 3 基于 5 个叶绿体基因序列片段(*psbA-trnH、trnG、trnL、trnL-F、trnT-L*)和 2 个核糖体基因序列片段(ETS、ITS)构建的娃儿藤亚族的系统发育树

Fig.3 Phylogeny tree of Tylophorinae constructed by five plastid markers (*psbA–trnH*, *trnG*, *trnL*, *trnL–F*, *trnT–L*) and two ribosomal gene sequences (ETS, ITS)

3 讨论与结论

3.1 秦岭藤属的系统发育与属级分类地位

在秦岭藤属建立时,Schlechter(1905)指出其花粉块下垂,与牛奶菜属(Marsdenia R. Br.)的直立花粉块存在明显差异;花冠坛状或近钟状,与娃儿藤属的花冠 5 深裂、辐状或广辐状有别;并认为秦岭藤属应放在马利筋亚族(Asclepiadinae)中。Gilbert 等(1995)认为秦岭藤属的特点是叶狭窄,叶正面具有微小的苍白点,花小,花冠缩小,通常呈环状,花冠管多数较发达,并观察到娃儿藤属的一些种也有相似的苍白点,说明秦岭藤属与娃儿藤属形态性状存在交叉现象。娃儿藤属已被归并至白前属中(Liede-Schumann et al., 2012;Liede-Schumann & Meve, 2018;Endress et al., 2018),本研究的分子系统结果支持秦岭藤属

也归并入白前属的分类处理。

分子系统分析结果显示,秦岭藤属是的 3 个种都在白前属内,其中秦岭藤与青龙藤聚为一支,而黑水藤与 V. villosum (T. villosa) 聚为一支,并在亚热带分支(Subtropical Clade)中,但它们在形态上差异较大,黑水藤的枝、叶柄均被单列短柔毛,叶线状披针形,除叶面中脉被微毛外,无毛,花冠近钟状,无毛,而 V. villosum 的枝、叶柄均密被柔毛,叶卵形或卵状长圆形,两面具毛,花冠辐状,具长柔毛;地理分布方面,前者分布于我国贵州、湖南、四川、西藏和云南(Li et al., 1995),而后者分布于爪哇岛(Backer & Bakhuizen, 1965)。有关黑水藤与近缘种在形态和地理分布上差异较大,但亲缘关系较近的情况,尚需进一步采集更多样品开展深入探究。

3.2 驼峰藤属的系统位置与属级分类地位

驼峰藤属在发表时被认为与徐长卿属(Pycnostelma Bunge ex Decne.)最相近(Chun & Tsiang, 1941),后者现已被归并至白前属中(Liede-Schumann & Meve, 2018),两者的副 花冠都贴生于雄蕊,但它们在生活型、副花冠裂片的形状、花冠裂片生长样式和果实形状大 小等方面存在明显区别,其中驼峰藤属为木质藤本、副花冠裂片背部隆起、基部增厚、花冠 裂片连生,未开花时裂片顶端向内连生、蓇葖果单生,纺锤形,大型;而徐长卿属为直立草 本、副花冠向先端加厚、花冠深裂、果实披针形,较小(Chun & Tsiang, 1941); 驼峰藤属 的花冠裂片与醉魂藤属(Heterostemma Wight & Arn.)和球兰属(Hoya R. Br.)类似,其膜 质心形叶片与夜来香属(Telosma Coville)相似,但驼峰藤属的花粉块下垂,另3个属的花 粉块直立,可以明显区分(Chun & Tsiang, 1941)。蒋英和李秉滔(1977)发现驼峰藤属与 印度产的原 Iphisia Wight & Arn=Vincetoxicum Wolf 较相近, 不同之处在于驼峰藤属的花冠裂 片端部未开放时互相粘合,花粉块柄斜行上升,且花粉块完全倒垂,而 Iphisia 的花粉块柄 先横平,次上升,后斜下。驼峰藤属的花部结构与宜昌娃儿藤[T. augustiniana (Hemsl.) Craib = V. augustinianum (Hemsl.) Meve & Liede]十分相似,但驼峰藤属的花比后者更大。此外,驼 峰藤属的蓇葖果大(长9~12 cm,直径3.5~4 cm),有较厚的纤维状中果皮,而娃儿藤属 通常果皮薄,具有纸质的蓇葖果壁,所以驼峰藤属被保留为独立的属(Gilbert et al., 1995)。 本研究未收集到宜昌娃儿藤的数据,但研究结果显示驼峰藤属与其他 2 个娃儿藤属 V. cissoides (T. cissoides) 和 V. philippicum (T. parviflora) 聚为一支,这 2 个种果实均较大(前 者长 10~12 cm, 直径 3~5 cm; 后者长 13~15 cm, 直径 3~4 cm), 叶大小与叶形与驼峰 藤属也很相似;地理分布上, V. cissoides 广泛分布于印度尼西亚东部和新几内亚(Forster, 1994), V. philippicum 仅分布于菲律宾(Meve et al., 2002), 而驼峰藤属分布于我国广东、 海南与柬埔寨(Li et al., 1995)。因此,仅凭果实的大小、花粉块柄的生长方式、叶子的形 状与大小作为娃儿藤亚族内属的鉴别特征可能是不够充分的,但这些特征可作为白前属内种 间的鉴别特征。依据花粉块下垂的特征, 驼峰藤属在最初建立时被置于马利筋族马利筋亚族 中 (Chun & Tsiang, 1941), 自 Endress 和 Bruyns (2000) 合并夹竹桃科与萝藦科后,该属 也被置于马利筋族中,但先后被置于鹅绒藤亚族(Endress et al., 2007)、娃儿藤亚族中 (Endress et al., 2014; Endress et al., 2018)。本研究支持当前驼峰藤属归并至白前属,并放 置在娃儿藤亚族中。

3.3 白前属的系统发育

虽然基于核糖体基因序列和叶绿体基因序列合并数据的系统发育分析显示白前属是单系的,但其含有的信息位点依旧较少,其属内的种间系统发育关系支持率较低。基于高通量测序的系统发育分析将更清晰地重建白前属的系统发育分支关系。白前属的分类变动较大,非常有必要基于基因组学研究结果与形态特征分析进行全面的分类学修订。

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